FIG._1A

FIG._1B

FIG._1C

FIG._1D

Integrin Heterodimer

αA Domain or αI Domain

βA Domain

β Propeller

PSI Domain

EGF-like Repeats

Plasma Membrane

α Domain

β Domain

FIG._1E

MALRVLLLTALTLCHGFNLDTENAMTFQENARGFGQSVVQLQGSRVVVGAP

QEIVAANQRGSLYQCDYSTGSCEPIRLQVPVEAVNMSLGLSLAATTSPPQL

LACGPTVHQTCSENTYVKGLCFLFGSNLRQQPQKFPEALRGCPQEDSDIAF

LIDGSGSIIPHDFRRMKEFVSTVMEQLKKSKTLFSLMQYSEEFRIHFTFKE

FQNNPNPRSLVKPITQLLGRTHTATGIRKVVRELFNITNGARKNAFKILVV

ITDGEKFGDPLGYEDVIPEADREGVIRYVIGVGDAFRSEKSRQELNTIASK

PPRDHVFQVNNFEALKTIQNQLREKIFAIEGTQTGSSSSFEHEMSQEGFSA

AITSNGPLLSTVGSYDWAGGVFLYTSKEKSTFINMTRVDSDMNDAYLGYAA

AIILRNRVQSLVLGAPRYQHIGLVAMFRQNTGMWESNANVKGTQIGAYFGA

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YGEQGQPWGRFGAALTVLGDVNGDKLTDVAIGAPGEEDNRGAVYLFHGTSG

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VNCSIAVCQRIQCDIPFFGIQEEFNATLKGNLSFDWYIKTSHNHLLIVSTA

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FIG._1F

```
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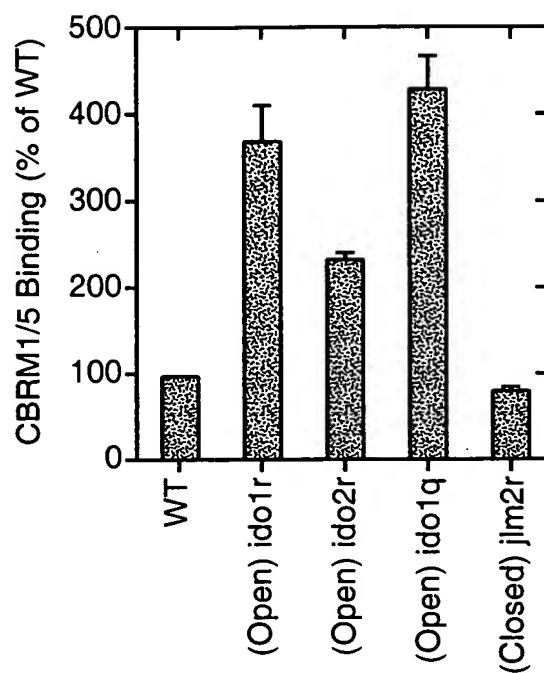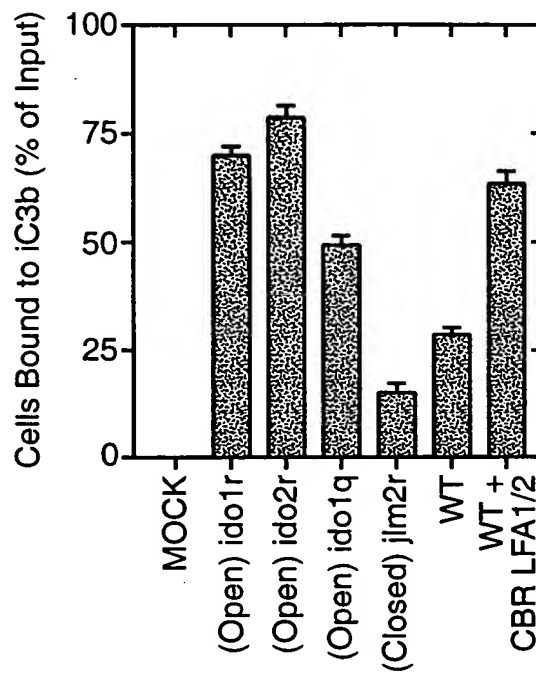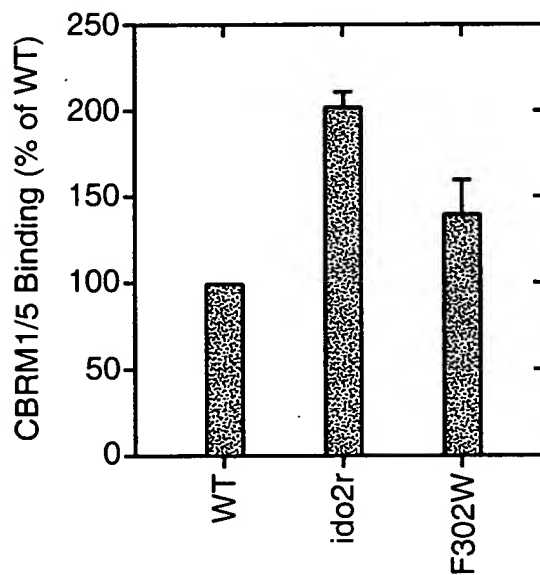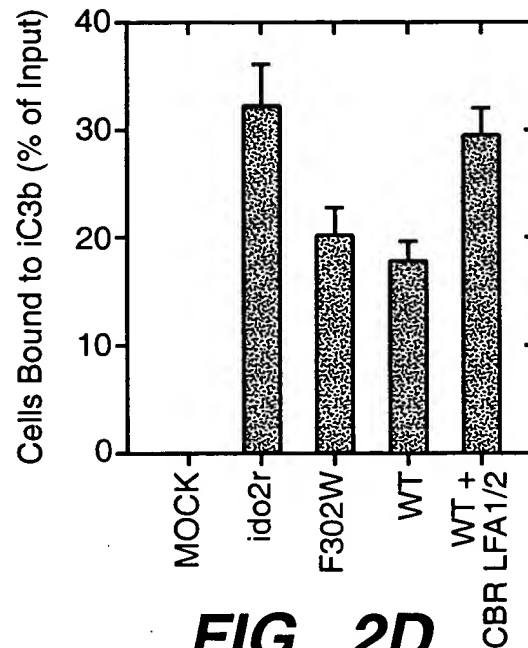
FIG._1G-1

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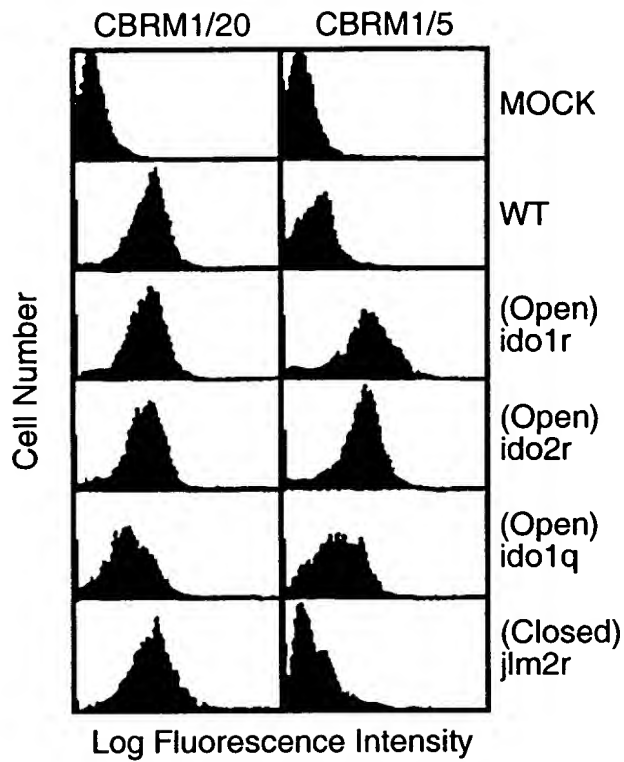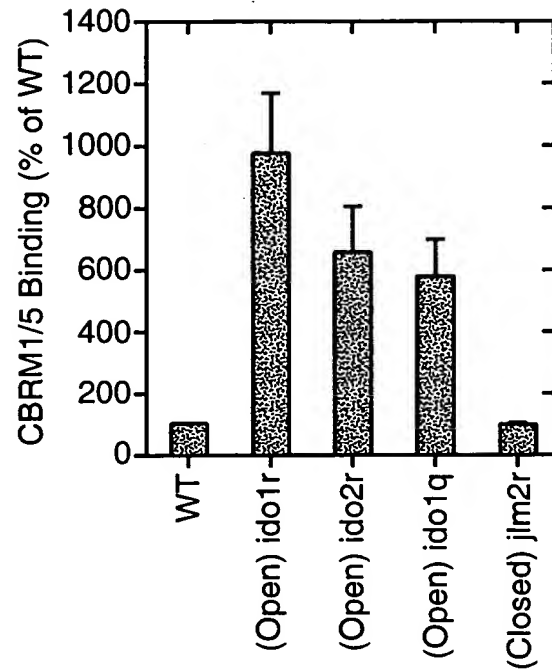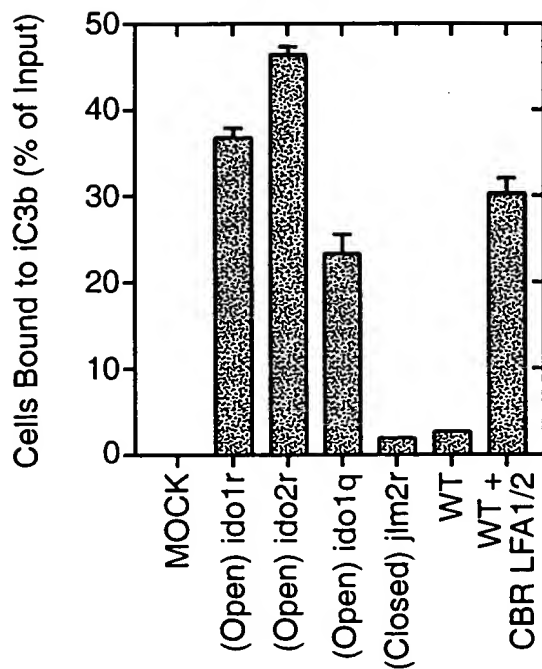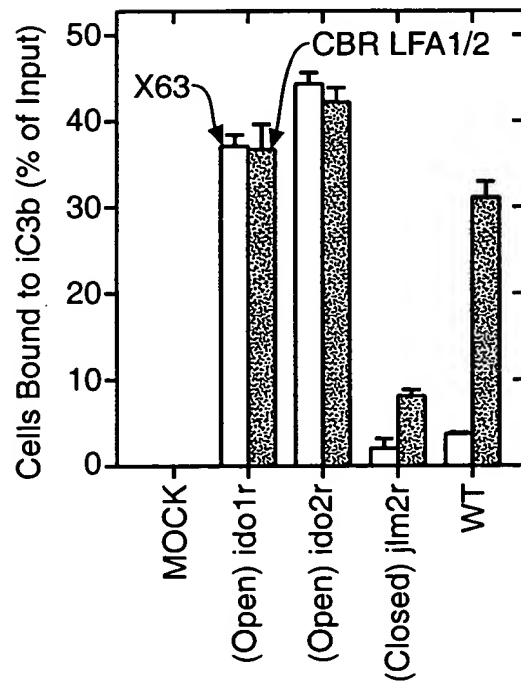
FIG._1G-2

FIG._2A

FIG._2B

FIG._2C

FIG._2D

FIG._3A

FIG._3B

FIG._3C

FIG._3D
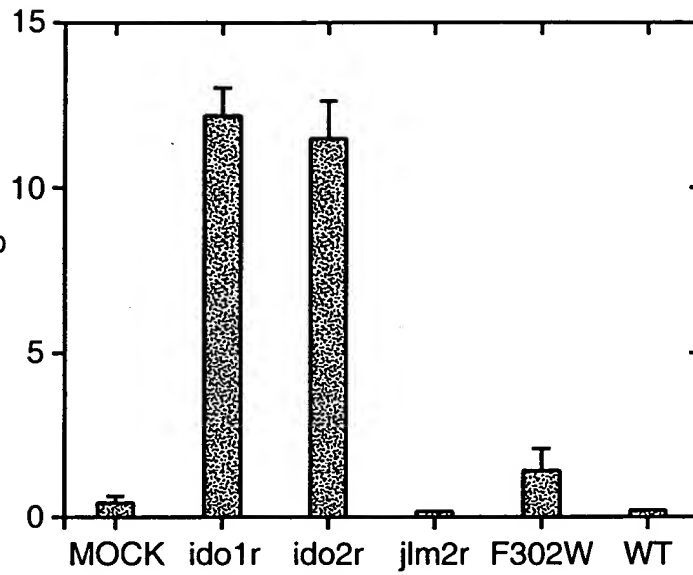
FIG._4A

FIG._4B

FIG._4C

09902481.040802

FIG._5

OLIGOS FOR
POSITIONS

OLIGOS FOR
POSITIONS

OLIGOS FOR
POSITIONS

PCR
PRIMER

WILD TYPE GENE
OR GENE FOR
GLOBAL MINIMA

PCR PRIMER

FIG._6

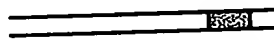BLACK BOX =
REGION TO
BE MUTATED

F1 →
F2 →
F3 →
TEMPLATE DNA
← R1
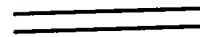← R2
← R3
← R4
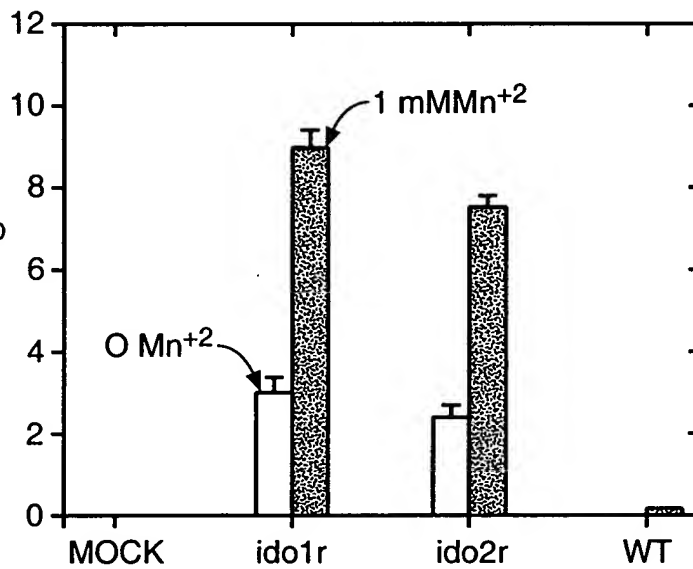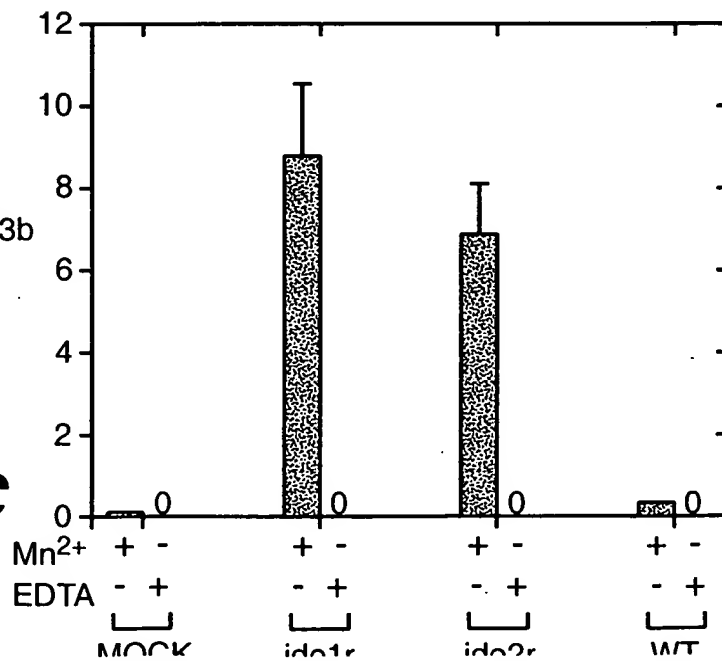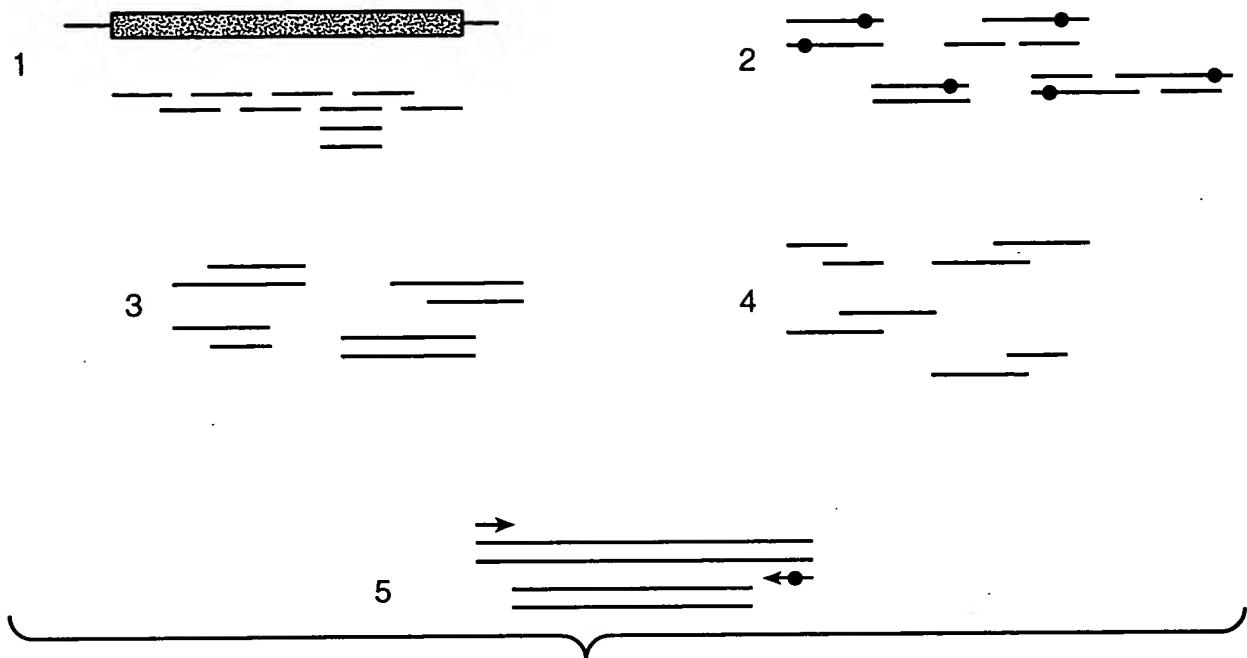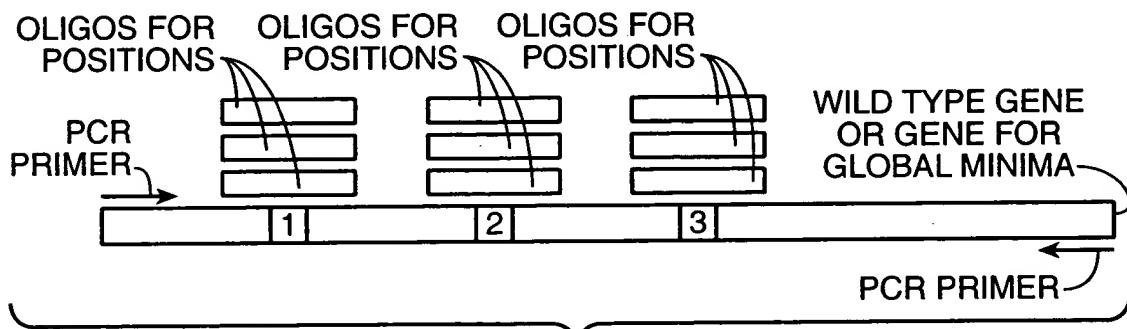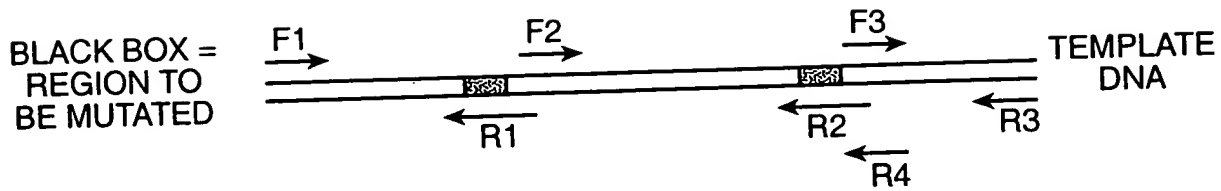
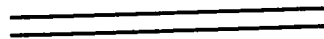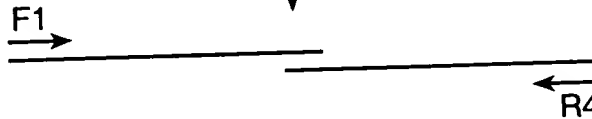STEP 1: SET UP 3 PCR REACTIONS:

PRODUCTS:

TUBE 1:

TUBE 2:
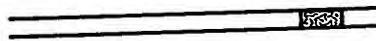
TUBE 3:

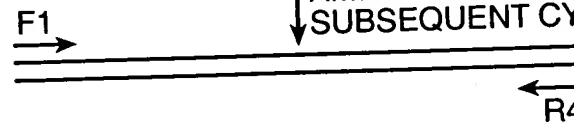STEP 2: SET UP PCR REACTION WITH PRODUCTS OF TUBE 1 + PRODUCTS TUBE 2 + F1 + R4.

↓ HEAT + ANNEAL PHASE OF PCR,

F1 →
← R4

↓ SYNTHESIS PHASE OF PCR,

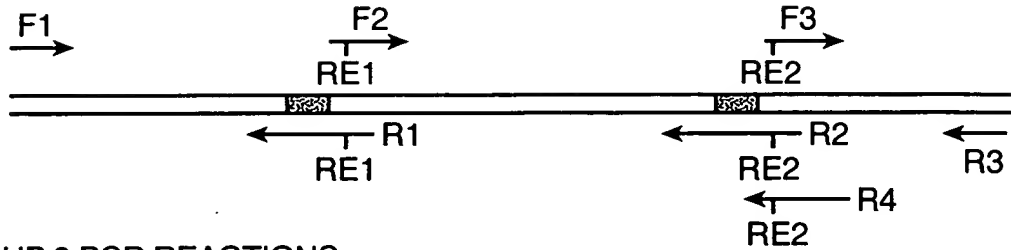↓ AMPLIFICATION PHASE DURING SUBSEQUENT CYCLES USING F1 + R4.

F1 →
← R4

STEP 3: REPEAT STEP 2 USING PRODUCT FROM STEP 2 + PRODUCT FROM STEP 1, TUBE 3 + PRIMERS F1 + R3.

FIG._7

A-70586-1

STEP 1: SET UP 3 PCR REACTIONS:

TUBE 1:

TUBE 2:

TUBE 3:

STEP 2: DIGEST PRODUCTS FROM STEP 1 WITH SUITABLE
RESTRICTION ENDONUCLEASES.

STEP 3: LIGATE DIGESTED PRODUCT FROM STEP 2, TUBE 2 WITH
DIGESTED PRODUCT FROM STEP 2, TUBE 1.

STEP 4: AMPLIFY VIA PCR LIGATED PRODUCTS OF STEP 3 WITH F1 + R4.

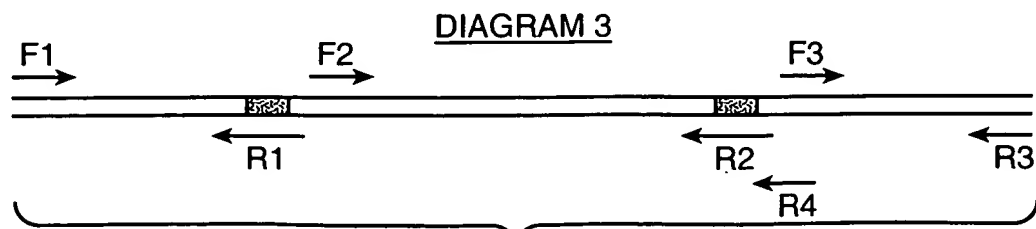STEP 5: DIGEST AMPLIFIED PRODUCT OF STEP 4
WITH RESTRICTION ENDONUCLEASE #2.

STEP 6: LIGATE PRODUCT FROM STEP 5 WITH
PRODUCT FROM STEP 2, TUBE 3.

STEP 7: AMPLIFY PRODUCT FROM STEP 6 WITH F1 + R3.

FIG._8

DIAGRAM 3